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14. ABSTRACT

Chronic pain is among the most prevalent health problems in the United States today, affecting 10% of the population and costing the U.S. billions of dollars each year in healthcare expenses, lost income, and lost productivity. Genetic differences among individuals in pain response physiology are partially responsible for observed variation in chronic pain development and maintenance. To identify genes affecting inter-individual variability in chronic pain response we are using a state of the art reference population of laboratory mice (Diversity Outbred mice). Diversity Outbred (DO) mice are a unique population of laboratory mice designed to maximize allelic variation throughout the genome (Churchill, Gatti et al. 2012). Each DO mouse is genetically unique. Unlike fully inbred strains, cohorts of DO mice approximate the levels of genetic (allelic) diversity found in human populations. The levels of segregating phenotypic and allelic diversity in DO mice allow for high precision for mapping regions of the genome that condition complex traits. Identifying genes whose allelic variants condition susceptibility to chronic pain development will further our understanding of the molecular mechanisms underlying chronic pain. This information, in turn, promises to facilitate improved methods for individualized chronic pain treatment and prevention.

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INTRODUCTION

The immediate goal of this study is to identify genes affecting inter-individual variability in chronic pain nociception using a state of the art reference population of laboratory mice (Diversity Outbred mice). The study design involves 1) using the formalin assay of nociception on a cohort of 300 Diversity Outbred (DO) mice, 2) genotyping the mice using the Mouse Universal Genotyping Array (MUGA), then 3) performing haplotype association mapping to identify regions of the genome that condition chronic pain response. Genes and other genome features that map to regions of the genome associated with chronic pain response will be analyzed using a variety of bioinformatics resources to predict candidate genes.

BODY

Activities during the first year were centered on tasks (1.1-1.8) associated with Objective 1 in the Statement of Work: "**Phenotype and perform genetic mapping analysis in a genetically diverse reference population of mice***: Phenotype a set of DO mice using the formalin test of nociception.*" No changes to the scope of the Statement of Work for Objective 1 were made. A Gantt chart showing tasks and timelines is shown below (Figure 1).

Progress on the tasks related to Objective 1 is described briefly below.

Task 1.1: Establish a phenotyping test environment (**Completed)**

A phenotyping platform was implemented that supports simultaneous phenotyping of 16 animals. Cameras are positioned under the testing chambers so that the entire test can be recorded and scored by multiple, independent observers to ensure statistical robustness of the data collected. The video feed from the cameras are captured directly to a hard drive.

Task 1.2: Obtain Diversity Outbred Mice **(Completed)**

We obtained a cohort of 300 non-sibling DO mice (150 males; 150 females). The animals were delivered in December 2011.

Task 1.3: Obtain DO founder lines **(Ongoing)**

Evaluation of phenotype data from DO mice is most informative when the phenotypes of the founder inbred lines are available. Phenotyping for five of the eight DO founder lines of mice has been completed. One of the strains (PWK) will be phenotyped in November of 2012. However, for the NZO strain it was not possible to obtain breeding stock and for the WSB strain, a breeding trio was obtained, but we were not successful in establishing a viable colony. During the next quarter we will purchase the remaining mice from the production colony at The Jackson Laboratory in order to expedite the completion of Tasks 1.3 and 1.6.

Task 1.4: Phenotyping refresher training **(Completed)**

All personnel received specific refresher training on the proper handling of mice for pain phenotyping from the Associate Veterinarian (Bonnie Lyons, DVM)

Task 1.5: Phenotyping of DO cohort mice **(Completed)**

Phenotyping of the DO cohort (150 male and 150 female mice) using the formalin assay for chronic pain was completed.

Task 1.6: Phenotyping of DO founder lines **(Ongoing)**

See explanation for Task 1.3 above.

Task 1.7: Genotype DO mice with the Mouse Universal Genotyping Array (MUGA) **(Scheduled)**

One of the primary tasks for the chronic pain gene discovery project is genotyping Diversity Outbred (DO) mice using a specially designed genotyping array (Mouse Universal Genotyping Array; MUGA). The MUGA is specifically designed for optimal genotyping of DO mice as it was designed using the genotypes of the 8 founder lines of the DO mapping population. The version of the MUGA array that we planned to use for our study at the outset is no longer available. Due to a delay in the release of the new Mouse Universal Genotyping

Array work on Task 1.7 is not complete. The new array was released in August 2012. DNA from the DO mice will be sent for genotyping by the end of September 2012.

Task 1.8: Perform QTL mapping **(Not Started)**

This task has not been started pending the completion of Task 1.7.

KEY RESEARCH ACCOMPLISHMENTS

The key research accomplishments for Year 1 include the following:

- Establishing the formalin phenotyping assay for measuring chronic pain in mice
- Successful completion of phenotyping methods refresher training for all study personnel
- Obtaining and phenotyping a cohort of 300 Diversity Outbred (DO) mice
- Obtaining and phenotyping 5 out of 8 DO founder lines
- Extracting DNA from 300 DO mice for genotyping

REPORTABLE OUTCOMES

None

CONCLUSION

With a few minor exceptions outlined above, we have successfully completed the specific tasks associated with Objective 1 in the Statement of Work for the Systems Genetics of Pain project and are on track for starting work related to Objective 2 (Candidate Gene Evaluation) on schedule as outlined in the original Statement of Work.

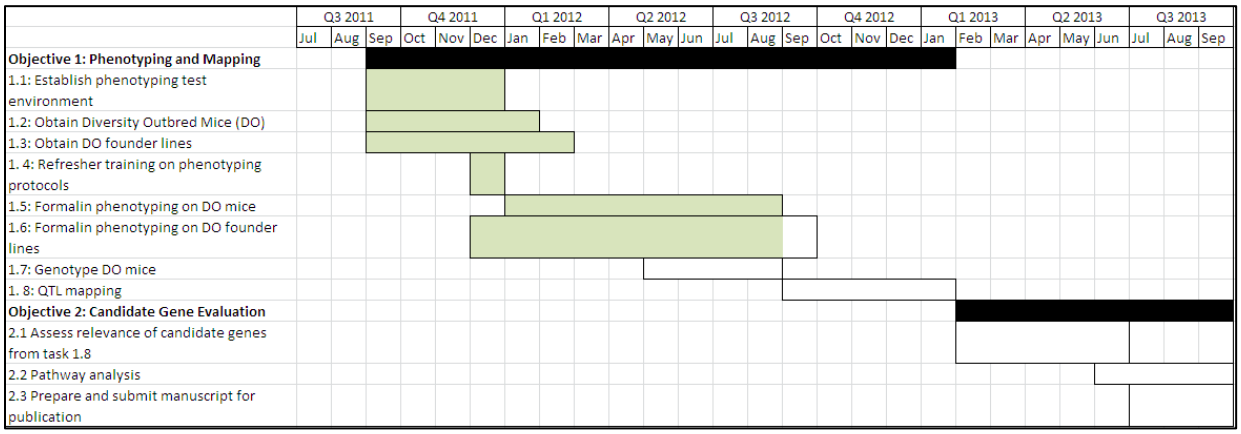
In the next phase of research activity we will perform genetic mapping using data generated for Objective 1 to identify genes related to chronic pain. We will also submit a manuscript describing the results of this study. Understanding the impact of genetic factors on chronic pain susceptibility will afford greater insight into the molecular mechanisms underlying injury-induced chronic pain in veterans, as well as the general population, facilitating the development of improved methods for prevention and treatment of chronic pain.

REFERENCES

Churchill, G. A., D. M. Gatti, S. C. Munger and K. L. Svenson (2012). "The diversity outbred mouse population." Mamm Genome.

APPENDIX

Figure 1. Gantt chart showing progress towards completion of tasks associated with Objective 1 of the Systems Genetics of Chronic Pain project (W81XWH-11-1-0762)



Gantt chart key:
Black line = Expected timeframe for objective
Solid gray/green = Task progress
Open = Task incomplete